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THE KARYOTYPES OF THE CHILEAN FROGS *EUPSOPHUS EMILIOPUGINI* AND *E. VERTEBRALIS* (AMPHIBIA: ANURA: LEPTODACTYLIDAE)

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Abstract.—The karyotypes of *Eupsophus emiliopugini* and *E. vertebralis* are described. Both species have 28 chromosomes, and *E. vertebralis* has a telocentric chromosome which is absent in *E. emiliopugini*. Two hypotheses about the karyological evolution of these frogs are proposed.

Eupsophus emiliopugini and *E. vertebralis* are endemic and allopatric frogs of the temperate *Nothofagus* forests of southern Chile (Formas 1989a). Bogart (1970) described the chromosomes of *E. vertebralis* based on two males collected at Mehuín (Valdivia Province), and pointed out that this species has 28 chromosomes, no detected secondary constrictions and a large telocentric (t) chromosome 3. *Eupsophus vertebralis* was considered by Donoso-Barros (1976) a synonym of *E. vittatus* (Philippi, 1902), however Formas (1989b) demonstrated that *E. vittatus* is a member of the genus *Alsodes* and *E. vertebralis* is a distinct species. *Alsodes vittatus* and *E. vertebralis* are allopatric species.

This paper describes the karyotype of *E. emiliopugini* and re-describes the karyotype of *E. vertebralis*. Two hypotheses are proposed to explain the karyological evolution of both species.

Methods and Materials

Specimens of *E. emiliopugini* were collected in Puntra (Chiloé Province) and *E. vertebralis* in Mehuín (Valdivia Province), the same locality where the specimens of *E. vertebralis* karyotyped by Bogart (1970) were collected. The numbers of individuals from each locality were: Puntra (42°7′S; 73°49′W) 4 males (IZUA 2058–2061) and 2 females (IZUA 2062–2063) and Mehuín (39°26′S; 73°10′W) 9 males (IZUA 1972–1979, 1983) and 1 female (IZUA 1981). All frogs were injected with 0.3 ml of colchicine solution (0.1%) for 12 hours, and chromosomes from intestinal cells were obtained. Fragments of intestine were hypotonically treated, fixed in acetic-alcohol (1:3), and placed in 45% acetic acid. Small fragments of tissue were squashed between two slides, dipped in liquid nitrogen, and the cover-slide was removed with a razor blade. After three days the slides were stained for 10 minutes in Sorensen's phosphate buffer (pH 6.8) containing 4% Giemsa. For karyotype analysis chromosome lengths were measured on enlarged photomicrographs of metaphase-spreads (18 mitotic plates from *E. emiliopugini* and 20 from *E. vertebralis*) and relative length and arm ratio (length of long arm/length of short arm) were calculated. The relative length of the chromosomes were determined according to Bogart (1970). The centromeric positions were determined according to Levan et al. (1964). Secondary constrictions were not included in the measurements.

Specimens and slide chromosomes were deposited in the amphibian collection of the Instituto de Zoología, Universidad Austral de Chile, Valdivia (IZUA).

Results

Eupsophus emiliopugini Formas, 1989

Examination of 18 metaphase plates from a total of 4 males and 2 females revealed a

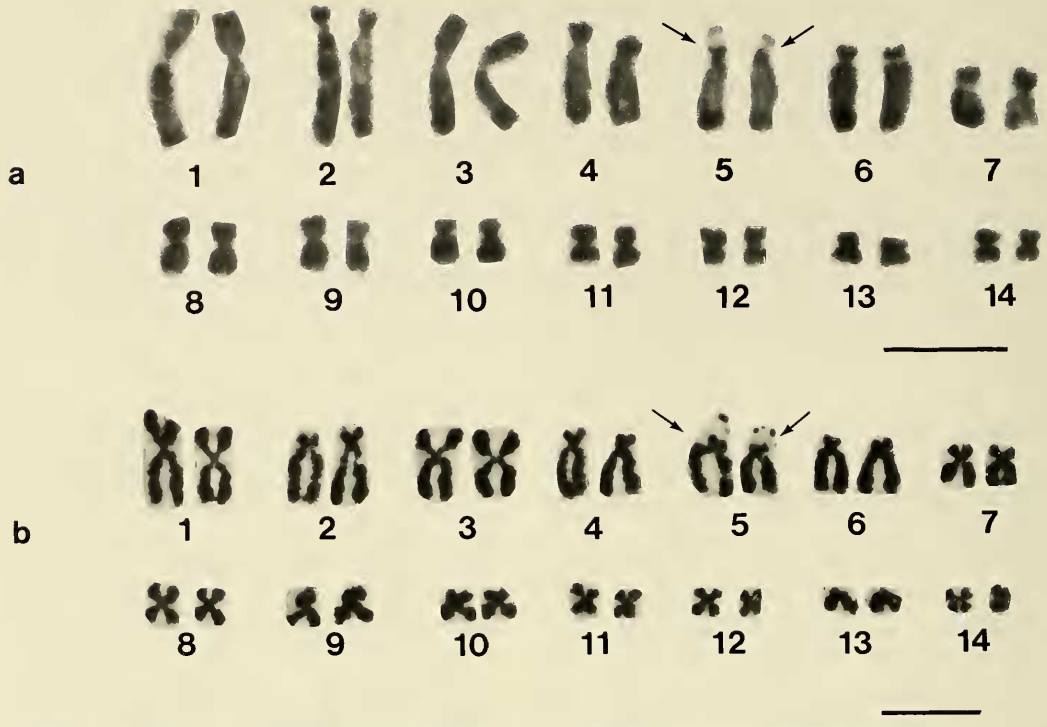

Fig. 1. Karyotypes of *Eupsophus emiliopugini* (a) and *E. vertebralis* (b). The bar indicates 10 μm.

diploid number of 2N = 28. All chromosomes are bi-armed and have a fundamental number (NF) of 56. When chromosomes are arranged in pairs of decreasing length: pairs 1–4 are large (>100 units), 5 and 6 intermediate (80–100 units), and 7–14 small (<80 units). Pairs 1, 3 and 8 through 14 are metacentric; 7 submetacentric, and 2, 4, 5 and 6 subtelocentric. The small arm of pair 5 shows an extended secondary constriction. The karyotype of *E. emiliopugini* is shown in Fig. 1a. No sexual dimorphism was detected in the karyotype. A summary of the centromeric positions and relative lengths of chromosome pairs is given in Table 1.

Eupsophus vertebralis Grandison, 1961

The karyotype of *E. vertebralis* is re-described from a total of 20 c-metaphase plates of 9 males and 1 female. The karyotype is shown in Fig. 1b. Pairs 1, 3, 8, 9, 10, 11, 12 and 14 are metacentric; 7 is submetacentric; 2, 4, 5 and 6 are subtelocentric, and pair 13 is telocentric. Pairs 1–4 are large (>100 units), 5 and 6 intermediate (80–100 units), and 7–14 small (<80 units). A summary of the centromeric positions and relative lengths of the chromosome pairs is given in Table 2. No sexual dimorphism was detected in the karyotype.

Discussion

The karyotype of *E. vertebralis* as described by Bogart (1970) differs in part when compared with the results here obtained. First, an evident secondary constriction was observed on the small arm of pair 5 (Fig. 1b). In addition, chromosome pair 3 was metacentric, not telocentric, as previously described. Finally, a telocentric not metacentric pair (13) was observed. The karyo-

Table 1.—Relative length, arm ratio (mean and standard deviations), and type of chromosomes (m = metacentric; sm = submetacentric; st = subtelocentric; t = telocentric) of *Eupsophus emiliopugini*. The number below males and females indicates the karyotypes measured. * Chromosome with secondary constriction.

	4 Males 12			2 Females 6		
Pair no.	Relative length	Arm ratio	Type	Relative length	Arm ratio	Type
1	140.81 ± 11.33	1.41 ± 0.17	m	139.90 ± 10.71	1.39 ± 0.35	m
2	122.80 ± 8.57	4.05 ± 1.44	st	122.84 ± 9.31	4.12 ± 0.94	st
3	115.24 ± 5.69	1.51 ± 0.14	m	116.01 ± 5.41	1.48 ± 0.23	m
4	107.33 ± 3.75	4.40 ± 1.38	st	108.25 ± 4.02	4.32 ± 1.12	st
5*	96.21 ± 9.21	3.60 ± 0.80	st	*96.18 ± 8.57	3.82 ± 0.79	st
6	88.27 ± 6.70	3.85 ± 0.58	st	87.93 ± 5.80	3.71 ± 0.23	st
7	61.34 ± 4.81	1.90 ± 0.24	sm	61.75 ± 4.92	1.98 ± 0.15	sm
8	54.30 ± 5.41	1.36 ± 0.20	m	53.83 ± 6.95	1.51 ± 0.39	m
9	47.39 ± 3.52	1.43 ± 0.22	m	46.80 ± 4.71	1.36 ± 0.79	m
10	44.23 ± 2.75	1.31 ± 0.29	m	44.61 ± 1.93	1.20 ± 0.24	m
11	36.38 ± 2.80	1.55 ± 0.28	m	35.95 ± 3.07	1.43 ± 0.15	m
12	31.01 ± 4.47	1.06 ± 0.08	m	31.24 ± 3.68	1.12 ± 0.09	m
13	26.57 ± 3.85	1.10 ± 0.20	m	26.14 ± 3.94	1.08 ± 0.14	m
14	21.35 ± 4.64	1.03 ± 0.08	m	21.21 ± 3.66	1.20 ± 0.14	m

types of *E. vertebralis* and *E. emiliopugini* differ in the morphology of pair 13, which is telocentric in *E. vertebralis* and metacentric in *E. emiliopugini*.

Menzies & Tippet (1976) and Bogart (1981) explained the chromosome evolution of some frog species (*Litoria infrafrenata* and *Eleutherodactylus planirostris*) by translocation and pericentric inversion events. In these cases, the chromosomal number remained constant, whereas the fundamental number and the morphology of the chromosomes showed differences. This situation is also seen in the karyotypes

Table 2.—Relative length, arm ratio (mean and standard deviations), and type of chromosomes (m = metacentric; sm = submetacentric; st = subtelocentric; t = telocentric) of *Eupsophus vertebralis*. The number below males and females indicates the karyotypes measured. * Chromosome with secondary constriction.

	9 Males 16			1 Female 4		
Pair no.	Relative length	Arm ratio	Type	Relative length	Arm ratio	Type
1	126.00 ± 7.72	1.46 ± 0.25	m	125.93 ± 4.42	1.52 ± 0.14	m
2	119.00 ± 6.60	4.01 ± 0.76	st	119.40 ± 2.31	3.91 ± 0.81	st
3	109.83 ± 4.57	1.50 ± 0.20	m	108.31 ± 4.43	1.37 ± 0.16	m
4	102.83 ± 4.11	3.65 ± 0.41	st	101.92 ± 3.12	3.87 ± 0.52	st
5*	95.66 ± 5.50	3.74 ± 0.21	st	*94.32 ± 4.57	3.94 ± 0.15	st
6	90.83 ± 4.79	3.90 ± 0.45	st	89.71 ± 4.11	4.11 ± 0.29	st
7	63.16 ± 2.48	2.00 ± 0.24	sm	63.00 ± 3.97	1.98 ± 0.13	sm
8	55.00 ± 2.89	1.23 ± 0.24	m	54.98 ± 2.14	1.01 ± 0.32	m
9	51.16 ± 2.99	1.26 ± 0.20	m	52.83 ± 3.81	1.16 ± 0.72	m
10	47.83 ± 3.54	1.38 ± 0.09	m	47.93 ± 4.69	1.46 ± 0.12	m
11	42.66 ± 3.72	1.10 ± 0.10	m	41.36 ± 3.73	1.27 ± 0.33	m
12	37.50 ± 5.08	1.10 ± 0.12	m	37.00 ± 2.04	1.24 ± 0.12	m
13	29.16 ± 7.02	∞	t	28.16 ± 1.35	∞	t
14	22.66 ± 6.68	1.01 ± 0.04	m	21.94 ± 4.41	1.15 ± 0.31	m

Table 3.—Summary of the information available about the karyotypes of the species of *Eupsophus*.

Species	2N	NF	No. biarmed chromosomes	No. telocentric chromosomes	Source
E. emiliopugini	28	56	28	0	This paper
E. vertebralis	28	54	26	2	(Bogart 1970). This paper
E. roseus	30	46	16	14	(Iturra & Veloso 1989)
E. calcaratus	30	46	16	14	(Formas 1980)
E. migueli	30	45 male 46 female	15 male 14 female	15 male 16 female	(Iturra & Veloso 1989)

of *E. vertebralis* and *E. emiliopugini*. Both frogs have the same chromosomal formula (2N = 28), but the fundamental number is different (*E. emiliopugini* 56 and *E. vertebralis* 54), and the morphology of pair 13 is different (telocentric in *E. vertebralis* and metacentric in *E. emiliopugini*). The following hypothesis is offered to explain the evolution of karyological difference between *E. vertebralis* and *E. emiliopugini*: there was a pericentric inversion in the telocentric pair 13, which shifted the centromere to a metacentric position in *E. emiliopugini*. The similarity in size of pair 13 in both species supports the inversion mechanism. Though an inversion in chromosome pair 13 is a reasonable hypothesis there is at least one alternative possibility.

King (1980) explained the chromosome evolution of some frog species (*Litoria meiriana, L. chloris, L. phyllochroa, L. pearsoni,* and *L. olongburensis*) by addition of heterochromatin. In these events the relative chromosome arm lengths are modified to the same degree as the amount of C-banded chromatin added. The best example is seen in *Litoria meiriana* where a polymorphism for a large telomeric addition of a block of heterochromatin occurs on pair 12. On the basis of these observations an alternative hypothesis for the evolution of the karyological differences between *E. vertebralis* and *E. emiliopugini* would be that the addition of a heterochromatic segment in the centromeric region of the telocentric pair 13 led to the metacentric pair 13 of *E. emiliopugini*. C-band data could be used to test both hypotheses or perhaps, to suggest alternative ones. Chromosomal data has been useful to establish relationships among frogs. Chromosomal similarities among the five karyotypically known *Eupsophus* species (Table 3), allow recognition of two groups: the *vertebralis* group (2N = 28) (*E. vertebralis* and *E. emiliopugini*) and the *roseus* group (2N = 30) (*E. roseus, E. migueli* and *E. calcaratus*). Species of *vertebralis* group species have few telocentric chromosomes (0–2), whereas those of the *roseus* group are characterized by having many (14–16). Within the *roseus* group, Iturra & Veloso (1989) demonstrated the existence of sex chromosomes in *E. migueli* and *E. roseus*. The presence of sex chromosome dimorphism in both species, compared to the isomorphism founded in *E. calcaratus, E. vertebralis, E. emiliopugini* and other lower telmatobines frogs, is probably apomorphic.

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